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Questions around mutation T1010I in *MET* gene: results of next generation sequencing in Polish patient with suspected hereditary adenoid cystic carcinoma

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Abstract. – OBJECTIVE: Adenoid cystic carcinoma (ACC) is a slowly growing cancer, which is the most common malignant tumor of the salivary glands. It is claimed that it is a non-inherited cancer. People with family history of ACC are reported extremely rarely. We present patients with suspected hereditary ACC.

MATERIALS AND METHODS: Next generation sequencing (NGS) was performed for both RNA and DNA isolated from FFPE material.

RESULTS: In DNA from tumor tissue we detected the mutation in *MET* gene, in exon 14 c.3029C>T (p.Thr1010Ile). It has never been proven that this mutation may play a role in the pathogenesis of ACC. The most important for our case report seems to be the patient's family history of cancer occurrence which indicates presence of familial cancer aggregation (familial cancer syndrome) and even familial lung cancer.

CONCLUSIONS: ACC is extremely rare; it is difficult to observe a specific genetic pattern and NGS can provide a lot of information about the genetic causes of this disease. Our work shows that the *MET* p.Thr1010Ile mutation can be associated with the hereditary occurrence of ACC.

Key Words:
Adenoid cystic carcinoma, Next generation sequencing, *MET* mutation.

Introduction

Adenoid cystic carcinoma (ACC) is a slowly growing cancer, which is the most common malignant tumor of the salivary glands. However, it could occur in other organs, including trachea or large bronchi. It is a very rare neoplasm diagnosed mainly in women aged 40-50[1-3]. Gawełko et al[4] reported that in South-Eastern Poland (around 2,100,000 inhabitants) in between 1990 and 2012 there were 6516 cases of malignant tumors of the head and neck region. Literature data indicate that among head and neck cancers ACC appears in only 1%. Based on these data, it can be estimated that in South-Eastern Poland, the ACC incidence was about 65 cases throughout 12 years. Furthermore, ACC is considered a non-hereditary malignancy and people with familial ACC are observed extremely rarely. Until now, there are a few reports[5,6] describing patients with familial inherited ACC, and exact data about the occurrence of ACC in Poland are not known.

Genetic abnormalities in adenoid cystic carcinoma are non-specific and could harbor changes in the following genes: *MYB* (*MYB Proto-Oncogene, Transcription Factor*), *ZFPM1* (*Zinc Finger Protein, FOG Family Member 1*), *LRIG1* (*Leucine Rich Repeats And Immunoglobulin Like Domains 1*), *CRIPAK* (*Cysteine Rich PAK1 Inhibitor*), *ZNF517, GARS* (*Glycyl-TRNA Synthetase 1*) and *DGKZ* (*Diacylglycerol Kinase Zeta*)[7-9].

We observed two cases of ACC in our Department of Pneumonology, Oncology and Allergology (Medical University of Lublin, Poland). In the first case described by us in 2005, ACC was observed as a tracheal polyp that imitated bronchial asthma in a 53-year-old woman[3].

Case Report

In the following case report, we present 43-years-old non-smoker woman with tumor of tracheal bifurcation resected in 2009, with histopathological confirmation of ACC. The patient was followed up until 2017, when in

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computed tomography (CT) numerous tumors in both lungs were presented. In the material from the wedge resection of one of the lesions (performed in December 2017), the recurrence of ACC was confirmed. The patient received four cycles of chemotherapy based on cisplatin and gemcitabine. In August 2019, progression of tumors in both lungs was observed in chest CT, and molecular profiling of tumor cells was requested in order to search personalized molecularly targeted therapies. Mutations in the *EGFR* gene, *ALK* gene rearrangement and expression of PD-L1 on cancer cells were indicated as negative and to evaluate a larger genetic landscape of the tumor, next-generation sequencing (NGS) was performed. DNA and RNA were extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue, which was resected in 2017, using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and RNeasy FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Quantity of nucleic acids was evaluated using Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). We used the Oncomine™ Focus Assay (Thermo Fisher Scientific, Waltham, MA, USA) allowing for simultaneous analysis of single nucleotide polymorphisms (SNPs), copy number variations (CNVs), substitution and INDEL changes in DNA, as well as genes rearrangements in RNA. Targeted sequencing allowed identification of abnormalities in selected 52 genes associated with different types of solid tumors. The libraries were prepared separately for both DNA and cDNA (transcribed from RNA) using the AmpliSeq™ Library Kit (Thermo Fisher Scientific, Waltham, MA, USA), and the yield of the libraries has been evaluated by Qubit4 Fluorometer. Prepared libraries were adjusted to working concentration 45pM ensuring optimal sequencing. Emulsion PCR was performed in IonChef (Thermo Fisher Scientific, Waltham, MA, USA) device, and ready libraries were loaded on Ion 520™ Chip (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed in the Ion Torrent semiconductor technology on the S5 sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Informed consent was obtained for genetic testing.

Sequencing data were analyzed in the Ion Reporter program (Thermo Fisher Scientific, Waltham, MA, USA). The missense substitution c.3029C>T (p.Thr1010Ile) in exon 14 of *MET* gene was detected in tumor DNA. In addition, we observed non-clinically significant SNPs c.4381A>G (p.Ile1461Val) and c.407C>T (p.Pro136Leu) in *ALK* and *FGFR4* genes, respectively.

Discussion

We focused on the *MET* gene mutation that occurs in the juxta membrane domain of *MET* gene. This domain encodes the fragment of hepatocyte growth factor receptor located between the cell membrane and the tyrosine kinase domain. This region may also contain following SNPs: p.Arg970Cys, p.Arg1009Ser, p.Ser1058Pro and exon 14 skipping mutation. Interestingly, every mentioned SNPs and mutations in *MET* gene except p.Arg1009Ser occur usually only in lung cancer, while p.Thr1010Ile may affect stomach, colon and ovarian cancers[10].

In ClinVar data base, p.Thr1010Ile substitution has a status "conflicting interpretations of pathogenicity" [https://www.ncbi.nlm.nih.gov/clinvar]. In spite of the undetermined status of that mutation, in many studies, it is described as benign, likely benign or mutation with uncertain significance. However, ClinVar or Varsome databases attribute this mutation to increased risk of papillary renal cell carcinoma or hereditary cancer-predisposing syndrome[11,12]. Therefore, p.Thr1010Ile mutation is reported as likely pathogenic in cancers, and its somatic or germinal nature cannot be judged[13,14]. Liu et al[15] described this variant as polymorphism connected with pathophysiology of breast cancer. Authors suggested that presence of this rare variant should be considered as a potential biomarker for qualification to anti-*MET* therapy in clinical trials[15]. Wasenius et al[16] reported missense p.Thr1010Ile mutation in 6 (6%) of the 104 thyroid cancer patients, and they indicated that the clinical and the molecular significance of this *MET* gene alteration is not known. However, some case reports about cancer patients with this mutation described p.Thr1010Ile as a germline mutation. Heeke et al[17] tested *MET* I1010T, in liquid biopsy using Oncomine cfTNA Panel and in an external testing center using the Foundation Liquid assay, in untreated late stage non-squamous lung carcinoma, however only the in-house test detected this mutation that was not a somatic but a germline polymorphism. These inconsistent data indicate that the role of p.Thr1010Ile mutation is not well clinically understood in cancer.

Adenoid cystic carcinoma is a rare tumor and this is the first case report of ACC patient with p.Thr1010Ile mutation in literature worldwide. Moreover, it has never been proven that this mutation may play a role in the pathogenesis of ACC. However, the most interesting for our case reports seems to be patient's family history of cancer occurrence. Maternal aunt and great-grandmother suffered from stomach cancer. While, the patient's father and both of his brothers suffered from lung cancer. Unfortunately, the pathomorphological diagnosis of lung cancer cannot be established today (patients have long been dead). Lung cancer has occurred in smokers, but the young age of this patients is noteworthy. The medical history of our patients indicates presence of familial cancer aggregation (familial cancer syndrome) and even familial lung cancer. Unfortunately, we do not know if p.Thr1010Ile mutation occurred in other patient's family members. We also wondered whether this mutation was inherited or arose *de novo* in our patient. Careful examination of the patient's family is advisable, but the patient withdrew her consent for further genetic testing.

Conclusions

Next-generation sequencing in patients with ACC may reveal genetic changes not previously observed in this disease. The performance of NGS for cases of adenoid cystic carcinoma is as important as for other cancers in terms of molecularly targeted therapies, but it can also show the genetic background of ACC. Our work shows that the *MET* p.Thr1010Ile mutation can be associated with the hereditary occurrence of ACC. Due to the fact that ACC is extremely rare, it is difficult to observe a specific genetic pattern and NGS can provide a lot of information about the genetic causes of this disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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